DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Bifenthrin PC Code No.: 128825

2. **TEST MATERIAL:** Bifenthrin technical Purity: 95.7%

3. **CITATION:**

Authors:	Picard, C.R.
Title:	10-Day Toxicity Test Exposing Midges (*Chironomus dilutus*) to Bifenthrin Applied to Formulated Sediment Under Static-Renewal Conditions Following OPPTS Draft Guideline 850.1735.
Study Completion Date:	June 30, 2010
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1037
Sponsor:	Pyrethroid Working Group Beveridge & Diamond 1350 I Street NW Washington, DC 20005
Laboratory Report ID:	13656.6143
MRID No.:	48593602
DP Barcode:	416798

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac

Signature: Christie E. Padova **Date:** 02/04/15

APPROVED BY: Teri S. Myers, Senior Scientist, CDM Smith

Signature: Teri S Myers **Date:** 06/03/15

5. **APPROVED BY:**

Signature: **Date:**

6. **STUDY PARAMETERS:**

Age of Test Organism:	3rd Instar, 11 days old
Definitive Test Duration:	10 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured sediment, bulk and OC-normalized

7. CONCLUSIONS:

Results Synopsis:

In terms of mean-measured sediment concentrations:

Survival:
LC_{50}: 429 µg ai/kg 95% C.I.: 245 to 1230 µg ai/kg
Slope: 0.906 (0.567 to 1.25)
NOAEC: 110 µg ai/kg
LOAEC: 200 µg ai/kg

Growth (AFDW):
EC_{50}: 185 µg ai/kg 95% C.I.: 149 to 231 µg ai/kg
NOAEC: 110 µg ai/kg
LOAEC: 200 µg ai/kg

In terms of OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 18,652 µg ai/kg TOC 95% C.I.: 10,652 to 53,478 µg ai/kg TOC
Slope: 0.906 (0.567 to 1.25)
NOAEC: 4783 µg ai/kg TOC
LOAEC: 8696 µg ai/kg TOC

Growth (AFDW):
EC_{50}: 8043 µg ai/kg TOC 95% C.I.: 6478 to 10,043 µg ai/kg TOC
NOAEC: 4783 µg ai/kg TOC
LOAEC: 8696 µg ai/kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: This study [is/is not scientifically sound] and is classified as [acceptable/supplemental (quantitative)/supplemental (qualitative)/invalid].

B. Rationale:

C. Repairability:

9. MAJOR GUIDELINE DEVIATIONS:

- Treatment level data were compared to the performance of the pooled control for all endpoints.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *H. azteca* or *Chironomus tentans*	*Chironomus dilutus* (formerly known as *C. tentans*)
Life Stage: For *C. tentans*: third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For *H. azteca*: 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	*C. tentans*: 3rd instar, 11 days old At study initiation, the head capsule width of a sub-population of 20 larvae ranged from 0.35 to 0.60 mm (mean of 0.44 mm), and the dry weight of a sub-population of 20 larvae averaged 0.21 mg/larvae.
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Environmental Consulting & Testing Superior, WI
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is	The midges were acclimated to the

Guideline Criteria	Reported Information
23°C. The test organisms should be cultured in the same water to be used for testing.	laboratory culture facility for 24 to 48 hours prior to testing. During the holding period, the dissolved oxygen ranged from 8.0 to 8.1 mg/L and the temperature was 22°C.
Feeding:	During holding and acclimation, the midges were fed a finely-ground flaked fish food suspension daily. On the first day of acclimation, the larvae were also fed 5.0 mL of *Ankistrodesmus falcatus*, a unicellular green algae.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality, disease or unusual behavior was observed in the test population 24 to 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged. Uncontaminated natural sediment is recommended.	Laboratory well water characterized as having a total hardness and total alkalinity as $CaCO_3$ of 64 to 78 mg/L and 19 to 24 mg/L, respectively, a pH range of 6.9 to 7.5 and a specific conductivity range of 410 to 460 µmhos/cm. Formulated sediment (Laboratory Batch No. 112509A) was prepared according to OECD Guideline 218 by mixing the following components (dw basis): 6.0% sphagnum peat moss, 20% kaolin clay, and 74% fine sand. While blending using a large-scale mixer, 6 L of laboratory well water was also added. Prior to use, the sphagnum peat was pre-soaked in dilution water for 5 days. During this time, the peat was amended with 142 g of powdered $CaCO_3$ to increase the pH from 3.2 to 6.0.
Does water support test animals without observable signs of stress?	Yes
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality.
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily: 22 to 24°C Continuous: 21 to 25°C
pH Should not vary more than 50%. Survival is best at pH >6.5 for *C. tentans.*	6.7 to 7.2

Guideline Criteria	Reported Information
Dissolved Oxygen Maintained between 40 and 100%.	3.7 to 8.2 mg/L (≥40% saturation)
Total Hardness Should not vary more than 50%. *H. azteca* are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	67 to 76 mg/L as $CaCO_3$
Conductivity Should not vary more than 50%.	390 to 400 µmhos/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 79% sand, 6% silt, 15% clay USDA Textural Class – sandy loam TOC – 2.3% Percent solids – 63.78% pH – 7.1 Ammonia concentration of pore water in controls at Day 0 – 2.2 to 2.3 mg/L (as N)
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	Not reported

Guideline Criteria	Reported Information
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	Bifenthrin technical Synonyms: FMC 54800 IUPAC name: 2-methylbiphenyl-3-ylmethyl (1*RS*,3*RS*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate CAS name: (2-methyl[1,1′-biphenyl]-3-yl)methyl (1*R*,3*R*)-*rel*-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate Description: Liquid Lot no.: PL08-0165 CAS No.: 82657-04-3 Purity: 95.7% Storage: room temperature in the dark Aqueous solubility: not reported
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	A 100-µg ai/mL primary stock solution was prepared by bringing 0.01046 g of test substance (0.0101 g ai) to 100 mL with acetone. Six individual dosing solutions were subsequently prepared (at 2.64, 5.12, 10.4, 21.4, 41.2 and 82.4 µg ai/mL) by diluting the appropriate amount of the primary stock solution into 25 mL acetone. All dosing solutions were clear and colorless, with no visible un-dissolved test substance. Negative and solvent controls were included in the test.

Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand and the solvent was allowed to evaporate off for 60 minutes. The dry sand was then added to 2.5 kg of wet sediment (total of 1.6445 kg dw) in individual glass jars. Each jar was then rolled for 4 hours at *ca*. 15 rpm. The jars were stored upright at 2 to 8°C for a 14-day equilibration period. Twice a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill for 2 hours at room temperature to ensure the sediment was homogeneous. The range of nominal concentrations (16 to 500 µg ai/kg dw) used in the definitive study was selected in consultation with the Sponsor, based on toxicity information obtained through preliminary testing.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	1. Glass and 40-mesh Nitex screen (for drainage) 2. 300 mL vessels containing 100 mL (*ca*. 4.0-cm layer) of sediment (equivalent to 91.3 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at *ca.* 275 mL.
Type of Dilution System Daily renewal or a flow-through system may be used.	Flow-through

Guideline Criteria	Reported Information
Flow Rate 2 volume changes/day	2 volume additions/day
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 530 to 960 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 10 mL per 1.6445 kg dw sediment The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of chironomid larvae (day -1), the test systems were established. Overlying water was gently added using a turbulence reducer, and each vessel was placed under the renewal system.

Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Midges were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten midges. The test was initiated when each intermediate beaker of midges was added to each respective test vessel.
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment**.**	<u>Preliminary toxicity assessment</u> • 10-day exposure at nominal levels of 0 (negative and solvent controls), 0.20, 2.0, 20, 200 and 2000 µg ai/kg • 9-day old larvae; three replicates per level, each containing 10 larvae • Survival averaged 97 (negative control), 90 (solvent control), 90, 90, 100, 77 and 0%, respectively • AFDW averaged 1.19 (negative control), 1.22 (solvent control), 0.88, 1.05, 0.89 and 0.38 mg per larva, respectively
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test vessels were observed daily for mortality and abnormal behavior.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 16, 31, 63, 130, 250 and 500 µg ai/kg dw
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 larvae per level, with 10 larvae per replicate vessel and 8 biological replicates per level An additional 6 replicates per level were maintained for chemical analysis and pore water quality and analysis.
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. *H. azteca* may be fed with a mixture of yeast, Cerophy., and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO. levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	Midges were fed a finely-ground flaked fish food suspension (4.0 mg/mL) once daily at a rate of 1.5 mL/vessel.

Guideline Criteria	Reported Information
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	Total hardness, alkalinity, specific conductance and ammonia were measured in each treatment level and control solution from a composite sample at Days 0 and 10. Dissolved oxygen (DO), temperature, and pH were measured in each replicate vessel on Days 0 and 10, and in one alternating replicate from each level on Days 1 to 9 (see Reviewer's Comments). In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature-controlled water bath.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Sediment from all levels was analyzed for bifenthrin on Days 0 and 10. Following removal of the overlying water, the sediment was centrifuged at *ca.* 1200 *g* for 15 to 30 minutes prior to analysis using GC-MS/NCI based on methodology validated at Springborn Smithers (see Reviewer's Comments section for further details).

11. <u>REPORTED RESULTS</u>:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Reviewer's Comments)

Guideline Criteria	Reported Information
Control Criteria Was control mortality ≤30%? Were control *C. tentans* an average size of ≥0.6 g?	 Negative control – 6% Solvent control – 15% Negative control – 0.84 mg/larva Solvent control – 1.01 mg/larva
Percent Recovery of Chemical:	Results of quality control (QC) samples fortified at 8.00, 65.0 or 500 µg ai/kg and analyzed concurrently with test samples: <u>Sediment:</u> 71.3 to 95.1% of nominal (n=5; excludes one outlier of 124%)
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	 - Survival - Ash-free dry weight (AFDW)
Raw data included?	Yes, sufficient

Effects Data

Toxicant Sediment Concentration, µg ai/kg dw		Survival % ± SD	Percent Inhibition (%)[a]	AFDW per Larvae (mg ± SD)	Percent Inhibition (%)[a]
Nominal	Mean-Measured				
Control	<LOQ[b]	94 ± 11	---	0.84 ± 0.21	---
Solvent Control	<LOQ	85 ± 13	---	1.01 ± 0.15	---
Pooled Control	---	89 ± 12	---	0.92 ± 0.20	---
16	13	88 ± 10	1.1	0.81 ± 0.16	12
31	23	78 ± 15	12	0.98 ± 0.24	-6.5
63	48	93 ± 12	-4.5	0.92 ± 0.22	0
130	110	84 ± 11	5.6	0.72 ± 0.26	22
250	200	68 ± 14*	24	0.28 ± 0.14[c]	70
500	400	34 ± 18*	62	0.22 ± 0.09[c]	76

[a] Relative to pooled control.
[b] LOQ = 1.2 to 1.3 µg ai/kg.
[c] Excluded from statistical analysis due to significant effect on survival at this level.
* Statistically-significant compared to the pooled control ($p<0.05$) based on Bonferroni's t-Test.

Biological:

After 10 days, survival averaged 94 and 85% for the negative and solvent controls, respectively, and 88, 78, 93, 84, 68 and 34% for the mean-measured 13, 23, 48, 110, 200 and 440 µg ai/kg treatment levels, respectively. Corresponding percent inhibitions (relative to the pooled control) were 1.1, 12, -4.5, 5.6, 24 and 62%, respectively. Differences were statistically-significant compared to the pooled control (89%) at the 200 and 440 µg ai/kg levels ($p<0.05$; Bonferroni's t-Test). Using mean-measured sediment concentrations, the NOAEC and LOAEC for survival were 110 and 200 µg ai/kg, respectively, and the 10-day LC_{50} (with 95% C.I.) was 350 (310 to 400) µg ai/kg.

Ash-free dry weight (AFDW) at Day 10 averaged 0.84 and 1.01 mg per larvae for the negative and solvent controls, respectively, and 0.81, 0.98, 0.92, 0.72, 0.28 and 0.22 mg per larvae for the mean-measured 13, 23, 48, 110, 200 and 440 µg ai/kg treatment levels,

respectively. Corresponding percent inhibitions (relative to the pooled control) were 12, -6.5, 0, 22, 70 and 76%, respectively. No statistically-significant differences were indicated compared to the pooled control (0.92 mg/larvae) at up to and including 110 µg ai/kg. Higher levels were excluded from statistical analysis of growth due to the significant effect observed on survival. Using mean-measured sediment concentrations, the NOAEC and LOAEC for growth were 110 and >110 µg ai/kg, respectively, and the 10-day EC50 (with 95% C.I.) was 160 (140 to 180) µg ai/kg.

Analytical:

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for bifenthrin. Recoveries in the stock solutions ranged from 74 to 99% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 63 to 110% of nominal concentrations.

Bifenthrin sediment concentrations were relatively constant during the 10-day study. For the nominal 16, 31, 63, 130, 250 and 500 µg ai/kg levels, Day-0 measured concentrations were 12, 23, 50, 120, 190 and 460 µg ai/kg, respectively, and Day-10 measured concentrations were 15, 22, 45, 100, 210 and 420 µg ai/kg, respectively. Mean-measured sediment concentrations were 13, 23, 48, 110, 200 and 440 µg ai/kg, representing 73 to 88% of nominal levels.

B. Statistical Results

Method: Statistical analyses were performed on midge survival and growth (ash-free dry weight, AFDW) using TOXSTAT® Version 3.5 statistical software. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. No statistical differences were indicated for either parameter, and treatment groups were compared to the pooled control data to determine potential treatment-related effects.

Data for both endpoints were tested for normality using the Chi-Square Test and for homogeneity of variance using Bartlett's Test. Survival and growth data met both assumptions and were subsequently analyzed using Bonferroni's t-Test. Survival data were analyzed before dry weight data, and any levels demonstrating a significant effect were excluded from subsequent analysis.

The NOAEC and LOAEC values were assigned based upon significance. All statistical analyses were conducted at the 95% level of certainty except in the case of the qualification tests (i.e., Chi-Square and Bartlett's Tests), in which a 99% level of certainty was applied.

Probit Analysis within TOXSTAT® was used to calculate the LC_{50} value with associated 95% confidence intervals (C.I.), while the linear interpolation method was used to determine the EC_{50} for growth.

Survival:
LC_{50}: 350 µg ai/kg 95% C.I.: 310 to 400 µg ai/kg
NOAEC: 110 µg ai/kg
LOAEC: 200 µg ai/kg

Growth (AFDW):
EC_{50}: 160 µg ai/kg 95% C.I.: 140 to 180 µg ai/kg
NOAEC: 110 µg ai/kg
LOAEC: >110 µg ai/kg

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed survival and ash free dry weight (AFDW) endpoints. For both endpoints, the negative and solvent control groups were compared using equal variance two-sample t-tests; no differences were detected and the treated groups were compared to the negative control. The data were tested for normality using Shapiro-Wilk's test and for variance homogeneity using Bartlett's test. Data for both endpoints satisfied parametric assumptions and the NOAEC and LOAEC were determined using Dunnett's test. The LC_{50} was determined using Probit linear regression and the IC_{50} for AFDW was determined using nonlinear regression. These analyses were conducted using CETIS v. 1.8.7.12 with backend settings implemented by EFED on 3/25/14. The results were expressed based on bulk sediment and OC-normalized mean-measured concentrations.

Survival:
LC_{50}: 429 µg ai/kg 95% C.I.: 245 to 1230 µg ai/kg
Slope: 0.906 (0.567 to 1.25)
NOAEC: 110 µg ai/kg
LOAEC: 200 µg ai/kg

Growth (AFDW):
EC_{50}: 185 µg ai/kg 95% C.I.: 149 to 231 µg ai/kg
Slope: N/A
NOAEC: 110 µg ai/kg
LOAEC: 200 µg ai/kg

In terms of OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 18,652 µg ai/kg TOC 95% C.I.: 10,652 to 53,478 µg ai/kg TOC

Slope: 0.906 (0.567 to 1.25)
NOAEC: 4783 µg ai/kg TOC
LOAEC: 8696 µg ai/kg TOC

Growth (AFDW):
EC_{50}: 8043 µg ai/kg TOC 95% C.I.: 6478 to 10,043 µg ai/kg TOC
Slope: N/A
NOAEC: 4783 µg ai/kg TOC
LOAEC: 8696 µg ai/kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's conclusions generally agreed with the study author's. Study results were provided in terms of mean-measured sediment (bulk and OC-normalized) in the Conclusions section of the DER. OC-normalized sediment concentrations were reviewer-calculated and rounded to two significant figures (the organic carbon content of the formulated sediment was 2.3%):

Nominal Sediment (µg ai/kg)	Mean-measured Sediment (µg ai/kg)	OC-Normalized Sediment (µg ai/kg OC)
16	13	560
31	23	1000
63	48	2100
130	110	4800
250	200	8700
500	400	17,000

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. It was also reported that previous studies performed at the laboratory indicated that only negligible amounts of pyrethroids partition to overlying water (Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112, Putt, 2005).

Day 0 and Day 10 pore water samples were analyzed using solid phase microextraction (SPME) by an external laboratory. In addition, the external laboratory was sent bulk sediment samples from the low and high treatment levels from Days 0 and 10 in order to generate pore water for analysis by SPME. Results of the SPME analyses were not included in this report, but were to be included in a supplemental report.

All exposure and QC sediment samples were analyzed for bifenthrin using gas chromatography with mass selective detection with negative chemical ionization (GC-MS/NCI) based on methodology validated at Springborn Smithers. The method validation

was conducted prior to the initiation of definitive testing and established an average recovery of 92.4 ± 10.8% from formulated sediment. In addition, a method extension was conducted to verify recoveries of bifenthrin in formulated sediment at a concentration of 2000 µg/kg. The method extension established an average recovery of 101 ± 8.25%. It was reported that conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation.

Measurements of pH in overlying water were inadvertently omitted on Days 1 through 3 in all treatment levels and controls. It was reported that throughout the remainder of the exposure period, pH measurements were within the expected range and control organisms met the protocol acceptability criteria. Consequently, this deviation did not have a negative impact on the results or interpretation of the study.

In addition to total hardness and specific conductivity, total alkalinity and ammonia were determined in the overlying water of each level on Days 0 and 10. During the study, total alkalinity ranged from 18 to 26 mg/L as $CaCO_3$ and ammonia (as N) ranged from ≤0.10 to 1.5 mg/L.

The redox potential, pH, total organic carbon (TOC), dissolved organic carbon (DOC), and ammonia (as N) were measured in a pore water sample obtained from each test level on Days 0 and 10. During the study, the redox potential ranged from 180 to 210 mV, the pH ranged from 6.7 to 7.0, the TOC ranged from 81 to 140 mg C/L, and the DOC ranged from 81 to 120 mg C/L. Ammonia levels ranged from 2.2 to 2.5 mg/L on Day 0 and increased to 7.8 to 9.5 mg/L on Day 10.

This study was conducted in compliance with all pertinent U.S. EPA GLP regulations (40 CFR, Part 160) with the following exceptions: routine water, sediment, and food contaminant screening analyses. These analyses, however, were performed using certified laboratories and standard validated methods.

The experimental test dates were January 15 – 25, 2010.

14. REFERENCES:

Adams, W.J., R.A. Kimerle, and R.G. Mosher. 1985. Aquatic safety assessment of chemicals sorbed to sediments. In: *Aquatic Toxicology and Hazard Assessment: Seventh Symposium* ASTM STP 854. R.D. Cardwell, R. Purdy, and R.C. Bahner, Eds. American Society for Testing Materials. 1985. pp. 429-453.

APHA, AWWA, WEF. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association, American Water Works, Association and Water Environment Federation, Washington, DC.

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Ditsworth, G.R., D.W. Schults, and J.K.P. Jones. 1990. Preparation of Benthic Substrates for Sediment Toxicity Testing. *Environmental Toxicology and Chemistry.* Vol. 9, pp. 1523-1529.

European Commission, 2000. Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. Working document. Directorate General Health and Consumer Protection. SANCO/3029/99 rev. 4, 11/07/00.

OECD. 2004. OECD Guideline for Testing of Chemicals. Sediment Water Chironomid Toxicity Test Using Spiked Sediment. Guideline #218. Adopted 13 April 2004.

Putt, A.E., 2005. Bifenthrin – Toxicity to Midge (*Chironomus tentans*) During a 10-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6106.

Putt, A.E., 2005. Bifenthrin – Toxicity to Estuarine Amphipods (*Leptocheirus plumulosus*) During a 28-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6107.

Putt, A.E., 2005. Cypermethrin – Toxicity to Midge (*Chironomus tentans*) During a 10-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6110.

Putt, A.E., 2005. Cypermethrin – Toxicity to Estuarine Amphipods (*Leptocheirus plumulosus*) During a 28-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6111.

Putt, A.E., 2005. Cypermethrin – Life-Cycle Toxicity Test with Midge (*Chironomus tentans*) During a 60-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6112.

Sokal, R.R., and F.J. Rohlf. 1981. *Biometry.* 2nd Edition. W.H. Freeman and Company, NY. 859 pp.

U.S. EPA. 2000. Office of Water. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. Test Method 100.4. EPA/600/R-99/064. March 2000. U.S. Environmental Protection Agency. Washington, D.C.

U.S. EPA. 2008. Ecological Effects Test Guidelines. OPPTS 850.1735 Spiked Whole Sediment 10-Day Toxicity Test, Freshwater Invertebrates. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. EPA 712-08-354.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, DC.

Weber, C.I., *et al.* (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd edition. EPA/600/4-89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

West, Inc. and David D. Gulley, 1996. TOXSTAT® Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

Zumwalt, D.C., *et al.* 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers. *Environmental Toxicology and Chemistry.* 13: 1311-1314.